MATERIALS AND METHODS NEEDED TO PREPARE AND SUBMIT MOSQUITO POOLS FOR ARBOVIRAL TESTING

Mosquitoes tested for West Nile virus (WNV) or any of the other arboviruses should be collected live and maintained in that condition until they have been identified and pooled; WNV degrades quickly in dead mosquitoes; any mosquitoes that are tested by the tissue culture method for virus isolation should be fairly fresh. To maintain the live virus in mosquitoes, they should be frozen soon after death. WNV will degrade and not grow on tissue cultures when it is in mosquitoes that have been dead and exposed to room temperatures for more than one day. After identification and pooling, it is ok to kill the mosquitoes by freezing. Materials needed for processing and pooling mosquitoes are as follows:

Item Description	Source *	Part Number
Triethylamine - (500 ml bottle)	Fisher Scientific	BP 616 500*
Disposable polypropylene culture tubes 12x75mm (1000. case) **Required for pools tested by the Norfolk Public Health Laboratory**	VWR Scientific	60818-281*
Plug type caps for tubes (1000/case) **Required for pools tested by the Norfolk Public Health Laboratory**	VWR Scientific	60819-070*
Fisherbrand Microfuge Tubes (2.0 ml conical screw cap tubes with caps and O-rings, sterilized (500 per case) **Required for pools tested by the State Division of Consolidated Laboratory Services (DCLS) Laboratory**	Fisher Scientific	Cat # 02-681-375

* Similar items may also be available from other suppliers.

Procedures: When possible, trapped mosquitoes should be returned to the laboratory alive. Trap bags or containers may be placed in a 48 qt, cooler chest so they do not become overheated in the vehicle after collection and during transport. Just prior to identification mosquitoes should be anesthetized with **Triethylamine (TEA)**. They can also be anesthetized by holding them in a closed cooler containing dry ice for 15 minutes. When using Triethylamine, trap bags or containers of live mosquitoes may be placed in a heavy-duty trash bag along with a cotton wad soaked with one bottle cap-full of Triethylamine. This operation should be performed outdoors in a well ventilated area, and rubber gloves should be worn to avoid dermal contamination with TEA. The trash bag should be held closed for approximately 8 minutes and then opened to check the condition of the mosquitoes. If some mosquitoes still have their wings buzzing, close the trash bag for an additional minute to achieve complete anesthetization. Anesthetization is an operation that requires precise timing and observation. An exposure of less than 8 minutes may not anesthetize mosquitoes sufficiently for sorting and identification. An exposure of 10 minutes or more may kill the mosquitoes. Some slight day-to-day, species-to-species variations may occur in the time required for anesthetization.

Anesthetized mosquitoes should be sorted, identified and pooled as quickly as possible. Mosquito pools should be made by **species**, **date collected**, and **location collected**. Each pool

identification label should contain a "Sample Number". If you are using the mosquito database (See Section B.5 of the Mosquito Surveillance Plan), the database will automatically create a Sample Number for each new pool entered into the database. Otherwise, the Sample number will appear as follows P-0784-HENR-29-03, with the P and the first four digits of the sample number being the pool number, the four letter code being the Surveillance Program ID [in this case it is HENR for the Henrico County program], the finally there is a two digit week number followed by a two digit year number. If you are not using the database which automatically numbers all entered pools, pools should be numbered consecutively (i.e., P-0001, P-0002, P-0003, P-0004,…etc.) starting from the beginning of each year (surveillance season).

Correct identification of all mosquitoes in a pool is important. Mosquito pools of most species should contain from 25 to 50 mosquitoes. Certain important vector species are difficult to trap in large enough numbers to pool, and others have been deemed important enough that they may be submitted in smaller pools of from 10 to 25 mosquitoes (see[1] in list below. Vials containing pooled mosquitoes should be placed in a freezer and held until shipment to the testing laboratory. Tubes should be shipped to the testing laboratory in an insulated container containing dry ice.

Mosquito surveillance organizations are allowed to submit as many pools per week as they can collect. However, if the laboratory is busy they may not be able to test more than 40 pools per week. Additional pools will be held by the laboratory until there is time to test them. Programs that submit more than 40 pools per week should include their priority species among the first 40 pools.

The species tested for WNV are listed in the table to the left.

Status

♦ Mosquito species that should be tested in pools of from 25 to 50 mosquitoes.

♦♦ Mosquito species that may be tested in pools of containing from 10 to 50 mosquitoes.

[1] Mosquito species that are important vectors and/or may be difficult to trap in large enough numbers to make pools of 25 mosquitoes.

[2] Damaged *Culex pipiens* and *Cx. restuans* that cannot be distinguished from each other during identification may be pooled as *Cx. pipiens/restuans,* or if *Culex* specimens are un-identifiable as any species they may be pooled as *Culex spp*.

Status	Mosquito Species
♦♦	*Aedes albopictus* [1]
♦♦	*Aedes vexans* [1]
♦	*Anopheles crucians*
♦	*Anopheles punctipennis*
♦	*Anopheles quadrimaculatus*
♦	*Culex erraticus*
♦♦	*Culex pipiens* [1, 2]
♦♦	*Culex restuans* [1, 2]
♦♦	*Culex salinarius* [1, 2]
♦	*Culiseta melanura*
♦	*Coquillettidia perturbans*
♦♦	*Ochlerotatus atropalpus* [1]
♦	*Ochlerotatus canadensis*
♦♦	*Ochlerotatus japonicus* [1]
♦	*Ochlerotatus infirmatus*
♦	*Ochlerotatus sticticus*
♦	*Ochlerotatus sollicitans*
♦	*Ochlerotatus taeniorhynchus*
♦♦	*Ochlerotatus triseriatus* [1]

Any surveillance program that strongly suspects West Nile virus transmission from a species that is not on the above WNV tested list may consult with Dr. David Gaines (VDH-Office of Epidemiology; [804] 786-6261), and/or consult with the laboratory manager in the labs that the pools are being submitted to obtain approval for testing that species.

Mosquito pools should be submitted either to Dr. Dee Pettit at the **Division of Consolidated Laboratory Services (DCLS)** State Laboratory in Richmond, or to the Dr. Karren Loftin, or Deepak Phaltankar at the **Norfolk Public Health Laboratory** in Norfolk: Mosquito surveillance programs working outside of the Tidewater Region Should send their pools and samples to the DCLS laboratory. Tidewater Region programs using the Norfolk Lab may originate from the following jurisdictions: Accomack Co., Chesapeake, Hampton, Isle of Wight Co., James City Co./Williamsburg, Newport News, Norfolk, Northampton Co., Portsmouth, Southampton Co./Franklin, Suffolk, Virginia Beach, York Co./Poquoson. To arrange for testing of mosquito pools contact the laboratory managers first. Contact information is as follows:

Division of Consolidated Laboratory Services
Attention: Dr. Dee Pettit
600 North 5th Street
Richmond, VA 23219
Tel. (804) 648-4480, Ext. 281

Norfolk Public Health Laboratory
Attention: Dr. Karren Loftin
830 Southampton Ave
Norfolk, VA 23510
Tel. (757)-683-2746

A paper copy of the mosquito database entry form for the submitted mosquito pools ("Pool Log") should be sent along with each shipment of pools. Electronic copies of the Pool Log file (in spreadsheet format) should also be e-mailed to the appropriate laboratory on the day that pools are sent. Database users should e-mail the "Pool Log" file as an Excel attachment. Each program should use a consistent file naming convention will help the laboratory identify the e-mailed files from each surveillance program. Mosquito surveillance programs not having access to computers or unable to use the Virginia Mosquito and Arboviral Tracking System database may copy and use form on the following page to accompany their submitted mosquito pools.

Weekly Laboratory Mosquito Pool Submission Form

Program ID [1]:	Program Name	
Date Shipped to Laboratory	Sample Dates: From_____ To_____	Week #

Pool #	Collection Date [2]	Collection Site ID [3]	County/City	Mosquito Species	Number in Pool	Trap Type	Sample Number [4]
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[1] Program ID = four letter code (e.g., Virginia Beach = VABE). To avoid duplication of Program Codes across jurisdiction, codes should requested and approved through Dr. Gaines at the VDH Office of Epidemiology (804-786-6261)

[2] Collection Date = mm/dd/yy

[3] Collection Site ID = two letter County/City code + four digit site number (e.g., VB-0012 would be Virginia Beach collection site #12; once used to describe a collection site, the Collection Site ID should be permanently assigned to that site and not be used again for another site (to create your own Site IDs, use the reference list for the two-letter County/City codes at the bottom of Attachment 3.D).

[4] Sample Number = the letter P + four digit consecutive pool number + the four letter Program ID code + two digit week number + two digit year number (e.g., P-0123-VABE-22-03 = Pool # 123 from the Virginia Beach Surveillance Program, collected on week# 22 of the year 2003).